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Characterization of antibiotic resistance genes in drinking water sources of the Douhe Reservoir, Tangshan, northern China: the correlation with bacterial communities and environmental factors

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Abstract

Background: Antibiotics and antibiotic resistance genes (ARGs) threaten ecological sustainability and human health, especially the drinking water sources of the Douhe Reservoir, which have critical significance amid their direct use by people and the ecological hub of flora and fauna. Although antibiotics and ARGs pollution in reservoirs have been reported, it is of no practical significance to only study the changes (increase or decrease) of the abundance and diversity of ARGs, and it is more important to explore the mechanisms of the changes affecting ARGs. Thus, the occurrence and prevalence characterizations of the spatial and seasonal of the ARGs, mobile genetic elements (MGEs) and bacterial communities were comprehensively studied in present study.

Results: 263 ARG (nine types of ARGs) and 51 MGE subtypes were detected in 56 samples, and the characteristics of the temporal and spatial distribution of ARGs, MGEs and the composition of bacterial communities were significantly different. Moreover, the correlation among the ARGs, bacterial communities, MGEs and environmental factors were defined, and the co-occurrence patterns associated with ARG subtypes, bacterial genera, and MGE subtypes between water and sediment of the Douhe Reservoir were different.

Conclusions: In summary, ARGs were ubiquitous presence in water and sediment of the Douhe Reservoir, and the multidrug, aminoglycoside and macrolide–lincosamide–streptogramin B (MLSB) were main types of ARGs. Bacterial genera and the environmental factors [such as temperature (T), nitrate–nitrogen (NO₃–N), total dissolved nitrogen (TDN), and total phosphorus (TP)] significantly affected the distribution pattern of ARGs. Overall, this research revealed the spatiotemporal change and transmission mechanisms of ARGs in the typical drinking water sources of reservoirs, which will supply clues to ensure the safety of water sources.

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Keywords: Douhe reservoir, Water and sediment, Antibiotic resistance genes, Mobile genetic elements, Bacterial community

Introduction

Hundreds of antibiotics have been isolated or synthesized and used in clinical medicine, agricultural planting, animal husbandry, aquaculture and other fields ever, since the penicillin was used to treat human infections in 1940s [1]. However, most of the different types of antibiotics used in various fields will eventually be released into the environment in an un-metabolized form through various approaches, such as agricultural and pharmaceutical wastewater, human and animal excrement. The continuous release of antibiotics into the aquatic environment will induce the appearance of antibiotic resistance genes (ARGs) [2–4]. For instance, many studies have found that different types of ARGs appeared in fish [5, 6], shrimp [7, 8], pigs [9], cattle [10, 11] and other animals worldwide due to excessive use of antibiotics in livestock and aquaculture. Similarly, studies have shown that ARGs were detected in all types of environments, such as the atmosphere [12], water [13], and soil [14, 15]. In particular, the occurrence and spread of ARGs in lakes and reservoirs will affect the bacterial community structures and functions in the water body, which would affect the biochemical cycle of the lakes/reservoirs and the migration, transformation and natural degradation of pollutants, and the aquatic ecosystems have been destroyed ultimately [16, 17]. Studies have proved that ARGs can persist in the environment and can be spread therein, relying on the mobile genetic elements (MGEs) (e.g., insertion sequence, integrons, and plasmids) by the horizontal gene transfer (HGT) among different bacterial communities, which may have more detrimental influence on the ecological environment and human health than the antibiotics themselves [15, 16]. In addition, the environmental factors such as temperature, nutrients and heavy metals can impact the HGT of sediment and bacterial communities in water. In Karst River (Guizhou), the environmental factors such as bicarbonate ions (HCO_3^-) and nitrate–nitrogen (NO_3^- -N) concentration are obviously correlated with ARGs and MGEs [16]. Heavy metals such as Cd and Cr are significantly correlated with ARGs in solid waste leachates in Shanghai [18]. In recent years, antibiotics and ARGs as new environmental pollutants have been extensively studied, because they pose a threat to human and environment [19–22].

The aquatic environment is an important source and sink of ARGs, which is highly susceptible to interference by human activities. Although the wastewater (including the antibiotics and ARGs) produced from human

activities such as clinical, pharmaceutical, breeding, industrial and agricultural activities has been treated by the sewage treatment plants (STPs) before discharge, many studies also have shown that the STPs cannot remove them altogether, which introduce a large amount of antibiotics and ARGs into the lakes/rivers ultimately [23, 24]. Therefore, a large number of drinking water source reservoirs at domestic and abroad are widely contaminated by antibiotics and ARGs due to the inadequate treatment of the upstream wastewater [25, 26]. For instance, in the Taipu River and Jinze Reservoir (Shanghai), it was reported that 283 ARGs were detected in all the samples, with an absolute abundance of 1.59×10^9 – 5.69×10^{10} copies/L [27].

With the continuous advancement of urbanization, high-density material flow and population have greatly accelerated the migration, and spread of antibiotics and their resistance genes in the environment. Therefore, reservoirs and the rivers between them can provide an ideal environment for the physical transmission, acquisition and dissemination of ARGs. These environments are closely related to the comprehensive influence between natural processes and human activities. In fact, there is no practical significance to only discuss the changes in the diversity and abundance of ARGs (increase or decrease). Differences in water and sediment environmental factors caused by seasonal hydrological gradients, such as total nitrogen (TN), total phosphorus (TP), dissolved oxygen (DO), and electrical conductivity (EC), may also affect the distribution characteristics of ARGs. Exploring the underlying causes behind variations of changes in ARGs is very important to control the occurrence and spread of ARGs to ensure the safety of the aquatic environment. However, there are few studies on the fate and related transmission mechanism of ARGs. Therefore, this study conducted a comprehensive investigation of ARGs in the water and sediment of the Douhe Reservoir and cascade reservoir group, Tangshan, northern China in summer (flood period) and winter (dry period) in 2019 using the quantitative polymerase chain reaction (qPCR). Technically, the high-throughput quantitative PCR (HT-qPCR) used in this study includes 381 primer sets (including 1 for 16S rRNA, 325 for ARGs and 55 for MGEs), covering almost all common major types of ARGs and MGEs to ensure obtain more comprehensive and reliable results for the ARGs in the Douhe Reservoir. Overall, this study investigated the occurrence and distribution characteristics of ARGs and MGEs in water and sediments in

different seasons of the Douhe Reservoir, and analyzed the correlations among the occurrence of ARGs and bacterial communities, MGEs and environmental factors to explore the factors influencing the occurrence of ARGs. This work will help to comprehensively understand the mechanism of occurrence and transmission of ARGs, which will benefit for the ecological sustainability and human health of drinking water sources of the Douhe Reservoir.

Materials and methods

Location selection and sample collection

The Douhe Reservoir belongs to drinking water sources of lake reservoirs, which is the only surface drinking water sources in Tangshan City (38°55′ to 40°28′, 117°31′ to 119°19′), northern China, and the supply of water to Tangshan City started since 1988. Especially, the Douhe Reservoir is not only the single surface water source of

drinking water in Tangshan City, but also the terminal regulating reservoir and water supply source of the "Luanhe Water to the Tangshan Diversion Project". The annual water consumption is about 235 million m³, and the annual water supply to Luanhe River is 300 million m³. The average precipitation of the Douhe Reservoir in the past 5 years was close to 600 mm. There are Panjiakou Reservoir, Daheiting Reservoir and Qiuzhuang Reservoir on the upstream water delivery channel of Douhe Reservoir. Among them, the Panjiakou Reservoir and Daheiting Reservoir are the main sources of the water supply project of the Luanhe River.

In this study, eighteen sampling locations were selected in the Douhe Reservoir and cascade reservoir group on the basis of the geographic and residential conditions of the Douhe Reservoir and its surroundings (Fig. 1). The GPS information of the sampling locations was summarized in Supplementary Information

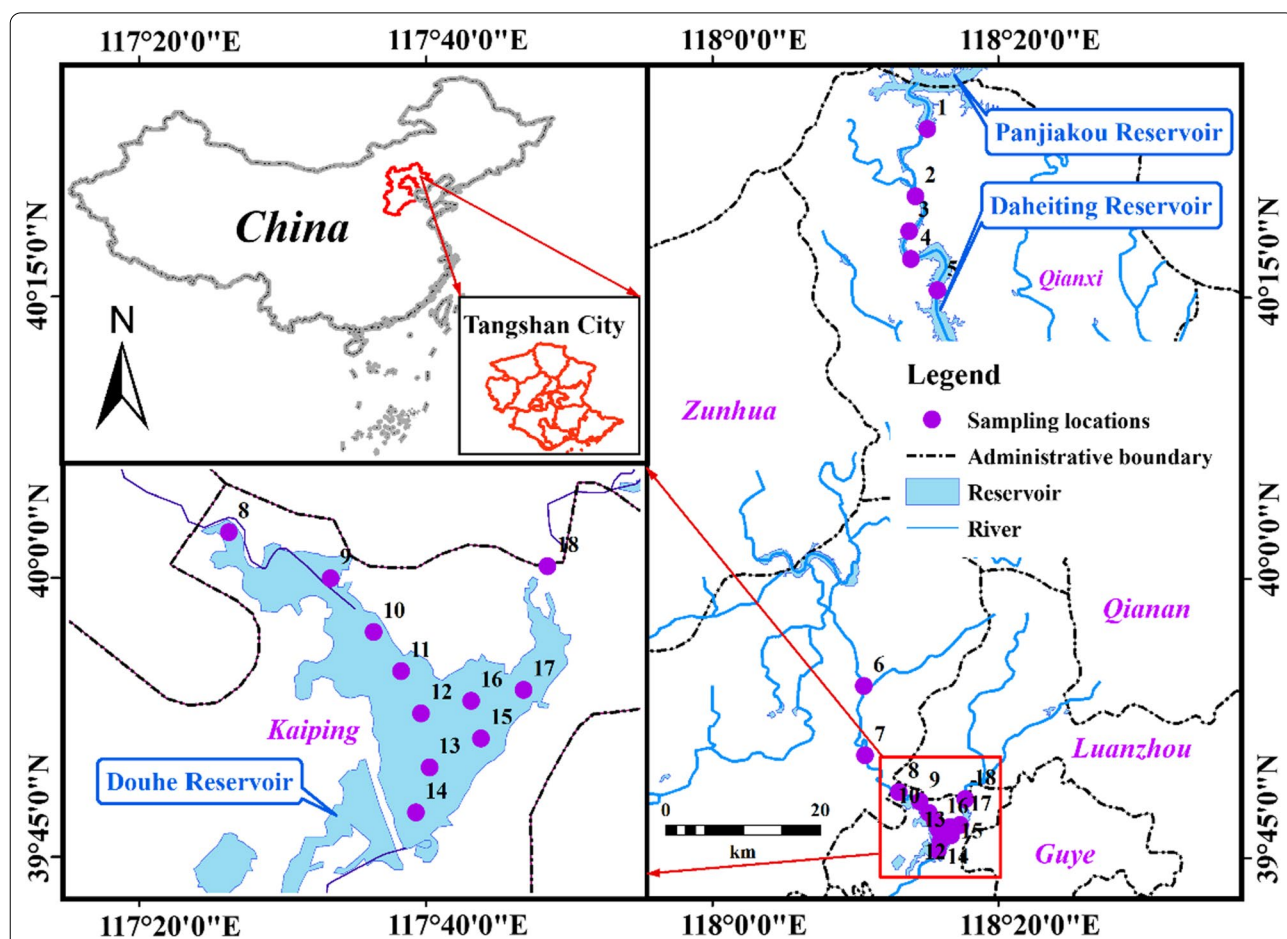


Fig. 1 Map of the sampling locations of the Douhe Reservoir and cascade reservoir group. Sampling location 1 is located in the Panjiakou Reservoir, sampling location 2 is located in the river channel between the Panjiakou Reservoir and Daheiting Reservoir, sampling locations 3–5 are located in the Daheiting Reservoir, sampling locations 6–7 are located in the river channel between the Daheiting Reservoir and Douhe Reservoir, sampling locations 8–18 are located in the Douhe Reservoir

(Additional file 1: Table S1). The survey for the Douhe Reservoir was performed in two seasons: summer (flood period, July 2019) and winter (dry period, October 2019), respectively. Eighteen water samples were collected from sampling locations 1 to 18 in the Douhe Reservoir and cascade reservoir group, and ten sediment samples were collected from the sampling locations 9 to 18 in the Douhe Reservoir area in each sampling campaign, respectively. Eighteen water samples of surface water (3 L, 0.5 m depth) were taken from all sampling locations and were placed in a pre-prepared clean brown glass bottles, and were stored in a refrigerator at 4 °C as soon as possible for processing within 24 h. For sediment samples, all collected samples (0–0.3 m depth) were kept in self-sealing plastic bag and stored at – 80 °C as soon as possible until analysis.

Environmental factors of water and sediment

Water quality parameters including DO, pH, temperature (T), Oxidation–Reduction Potential (ORP), EC were determined in the field using an analysis instrument (YSI ProDss, USA), and the details of the analysis methods for other physicochemical factors (including ammonium nitrogen ($\text{NH}_4\text{-N}$), $\text{NO}_3\text{-N}$, Pb, Cu and As, etc.) of sediment and water were listed in the previous study [15]. Analysis results are shown in Additional file 1: Tables S2 and S3, respectively.

DNA extractions and quantitative PCR amplification

Microorganisms in each water sample were enriched by filtering 1 L of water through a 0.22 µm membrane (Millipore, USA), and then the filter membrane was cut into pieces (2 mm × 2 mm) to extract DNA in water, and 1 g of fresh sediment was used to extract DNA in each sediment sample. EZNA™ Soil DNA kit (OMEGA, USA) was used for DNA extraction. The quality and concentration of the DNA extract of the samples were verified by spectrophotometry (Thermo Fisher Scientific, USA) and agarose gel electrophoresis (1%), and then the qualified DNA was adjusted to 50 ng/mL. Three DNA extracts were obtained for each sample, and all the sample DNA extracts were stored at – 80 °C until PCR analysis. All the profiles of ARGs and MGEs were determined by HT-qPCR using a Quantus™ Fluorometer (Promega, USA). There are 381 primer sets used in this research (including 325 for ARGs, 55 for MGEs, and 1 for 16S rRNA gene) [28–30] and the detailed information about each ARG and MGE is listed in Additional file 1: Table S4.

High-throughput illumina sequencing

In this study, high-throughput Illumina sequencing (Majorbio, Shanghai, China) was used to characterize the composition of bacterial communities in the water and sediment samples collected from the Douhe Reservoir, which was described in the previously study [28, 31]. Briefly, the PCR reaction was used to characterize the bacterial communities on the V4–V5 regions of 16S ribosomal RNA gene, and each reaction was performed in a 20 µL mixture, and the details of the PCR amplification conditions were consistent with the previous research [32]. To minimize the potential PCR bias of all the samples, the triple PCR reactions were implemented and purified, and then all purified products were adjusted to the same concentration and then mixed uniformly. Finally, the mixtures were sequenced on the MiSeq platform (Illumina, San Diego, USA). The chimeric sequences were removed after identification, and the operational taxonomic units (OTUs) with 97% similarity were clustered using UPARSE (usearch8.0.1616) [33]. The NCBI Sequence Read Archive (SRA) database was utilized for depositing the raw reads (Accession Number: SRP291227).

Statistical analysis

The ArcMap10.3 was used for visualization of the information for sampling locations. For the HT-qPCR data, all statistical analyses were performed using Excel 2017, and the difference in the occurrence and distribution of ARGs and MGEs were compared by software SPSS 20, and the results were considered significant at the 5% level ($p=0.05$). Origin 2017 was used to produce the bar chart and the boxplot. Meanwhile, the heatmap of the composition of the bacterial community was analyzed with R (version 3.5.2). The nonmetric multidimensional scaling (NMDS) analysis and the Adonis test were used to reveal the difference of the composition of bacterial communities between water and sediment samples collected from the Douhe Reservoir. The redundancy analysis (RDA) was applied to reveal the relationship between the ARGs, MGEs and bacterial community using Canoco 5.0 software. The ordinary least squares (OLS) was applied to analyze ARGs and MGEs through 'basic Trendline' package. Finally, the visualization of the network analysis was performed using the Gephi (version 0.9.1, <https://gephi.org/>) software.

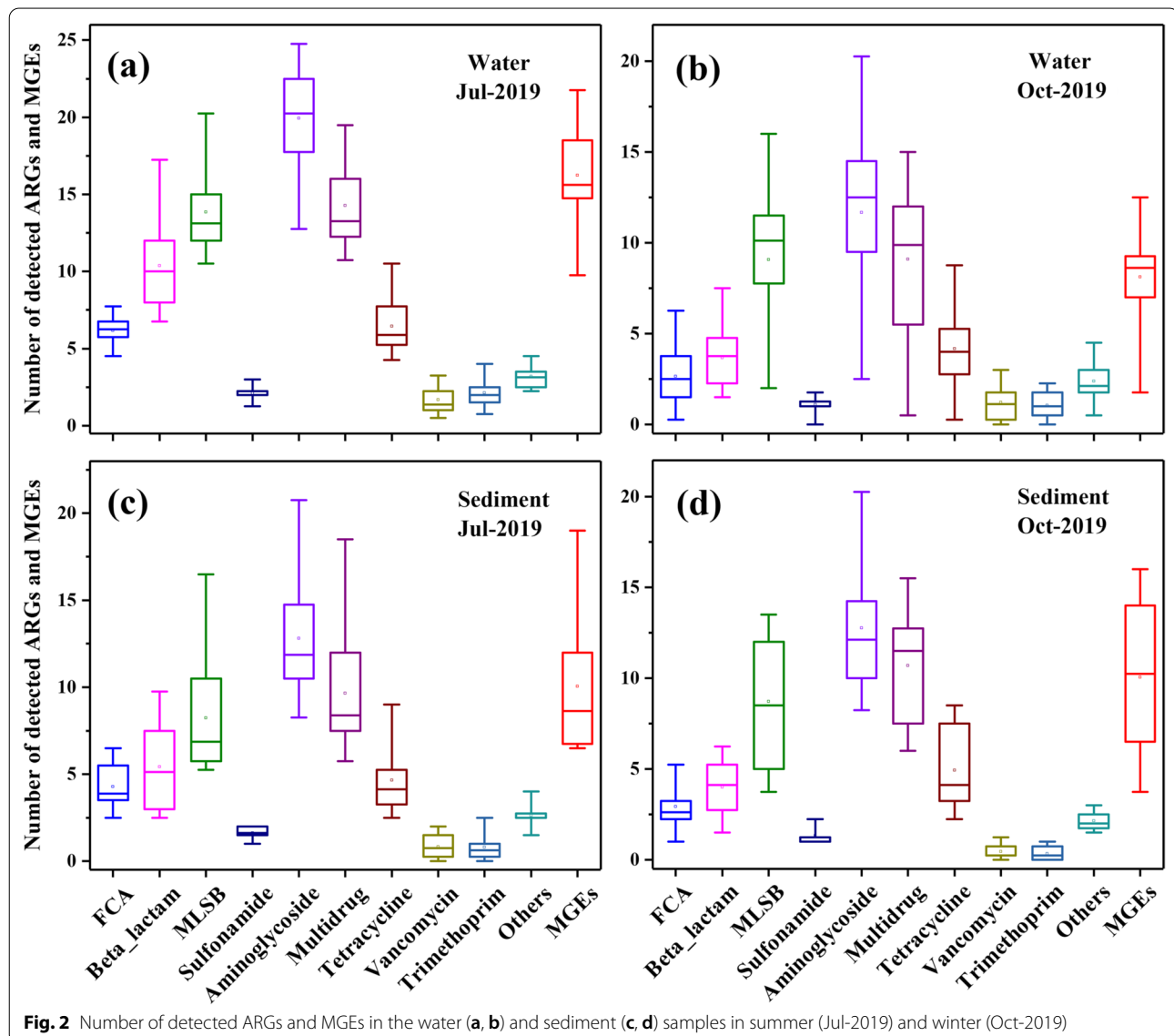
Results and discussion

Occurrence of ARGs and MGEs in the water and sediment samples

Diversity of ARGs

In all samples of the Douhe Reservoir and cascade reservoir group, there are 9 major types of ARGs detected (details data are given in Additional file 1: Table S5), which included trimethoprim, vancomycin, sulfonamide, tetracycline, MLSB (macrolides–lincosamides–streptomycin B), aminoglycoside, multidrug, FCA (fluoroquinolone, quinolone, florfenicol, chloramphenicol, and amphenicol), Beta-lactam resistance genes (Fig. 2). A total of 263 out of the 325 ARG subtypes were detected in the current study (261 were detected in water samples, including 250 and 197 subtypes in

samples of summer and winter, respectively, Fig. 2a, b, 200 were detected in sediment samples, including 179 and 163 subtypes in samples of summer and winter, respectively, Fig. 2c, d), which indicated that the water and sediments of the Douhe Reservoir and cascade reservoir group have been extensively polluted by ARGs. In fact, it is not surprising that ARGs are ubiquitous in fresh surface drinking water sources, since intrinsic antibiotic resistance also occurs in natural ecosystems, such as forest biomes with less human disturbance [34], Siberian permafrost and Beringian stage permafrost deposits 30,000 years ago [35]. Although ARGs are generally believed to be ancient and widespread, each ecosystem contains different bacterial communities and different dominant ARGs. Meanwhile, drinking



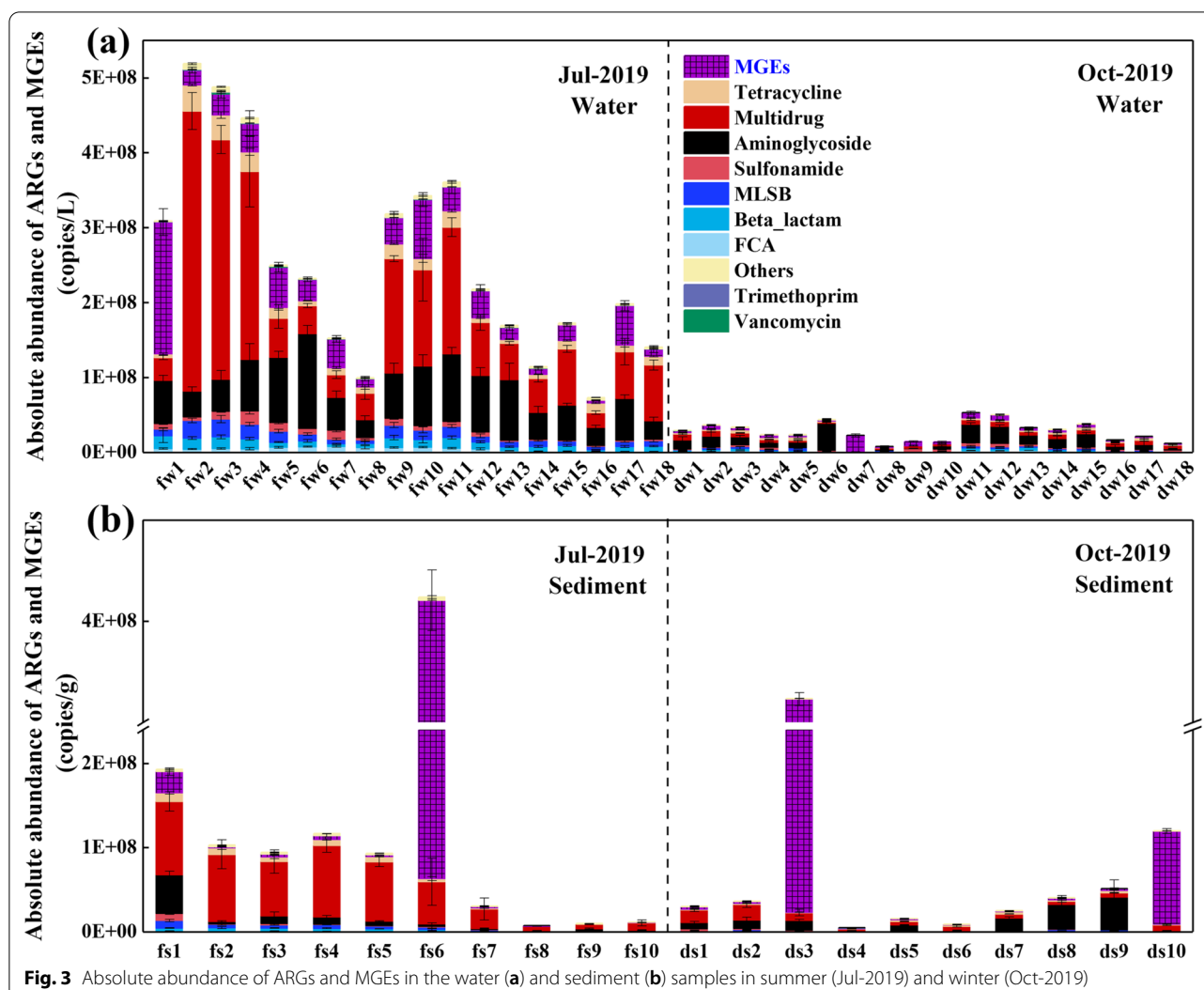
water sources are inevitably disturbed by human activities, and the influencing factors of ARGs may be more complex and diverse. This result about the number and types of detected ARGs in this study was similar with that in many previous studies for rivers, lakes and STPs [16, 28, 32, 36, 37]. The total number of ARGs detected in water and sediment samples of summer and winter of the Douhe Reservoir and cascade reservoir group are shown in Fig. 2, and the detailed data are given in Additional file 1: Table S6.

Among all the detected ARGs, the number of ARG of fw (fw1–fw18, water samples in flood period) were highest, ranging from 62.75 to 108.75, with a mean of 80.13, and followed by the number of fs samples (fs1–fs10, sediment samples in flood period) (32.50–86.50, mean of 50.90) and ds samples (ds1–ds10, sediment samples in dry period) (28.25–74.25, mean of 48.35). The lowest number of ARGs were in dw (dw1–dw18, water samples in dry period), ranging from 10.50 to 80.75, with a mean of 45.97. The results indicated that the total number of ARGs in water samples of the two seasons were significantly higher (One-way ANOVA, $F=4.85$, $p<0.05$) than those in sediment samples of the two seasons. At the same time, seasonally, the total number of detected ARGs in the water of summer was significantly higher (One-way ANOVA, $F=35.34$, $p<0.01$) than in the water of winter. This result was consistent with the results of the Karst River and Ebinur Lake (Xinjiang) [16, 38]. There are many types of resistance genes associated with antibiotics. Therefore, the selective pressure of antibiotics on microbial may be responsible for the production of ARGs [16]. As anthropogenic pollutant, evidence suggests that the movement of people and substances enhances the dispersal and proliferation of resistant organisms (including bacteria) between environments, so the type of antibiotic use and regional transport may have an impact on the distribution of resistance genes, and may lead to differences in the abundance and diversity of resistance genes [39]. In addition, higher water temperature in summer is favorable for bacterial activities, which promotes the occurrence of ARGs, resulting in significantly higher numbers of resistance genes detected in summer than in winter. In contrast, there was no significant difference in the total number of detected ARGs of water and sediment samples from the two seasons (Welch t test, $p>0.05$). Basing on the number of detected ARGs, the aminoglycoside (20.32–36.94%, average of 25.81%), multidrug (4.76–26.87%, 19.31%), MLSB (9.91–24.46%, 17.74%), Beta-lactam (4.64–16.51%, 10.23%) and tetracycline (1.49–12.55%, 8.62%) resistance genes were the five main types of ARGs in water and sediment samples of the Douhe Reservoir (Additional file 1: Table S6). The surveys of dominant ARG classes in the river-reservoir

system of Guangdong and Jiangxi Provinces (including Beiling River, Xunwu River and Fengshuba Reservoir) also showed similar results with this current research [32]. All the detected ARGs in this study were divided into 3 classes according to the resistance mechanism, in which antibiotic deactivation (average of 50.15%) were the most, followed by cellular protection (26.15%) and efflux pump (18.77%), respectively (Additional file 1: Table S4).

Abundance of ARGs

The total absolute abundance of 16S rRNA in each sample ranged from 2.50×10^8 to 2.48×10^{10} copies/L (water) and 9.14×10^8 to 5.34×10^9 copies/g (sediment); the total absolute abundance of ARGs ranged from 4.70×10^6 to 5.16×10^8 copies/L (water) and 4.95×10^6 to 2.39×10^8 copies/g (sediment). The absolute abundance of the nine major types of ARGs are shown in Fig. 3, and the detailed data are given in Additional file 1: Table S5. It can be observed that the absolute abundances of ARGs and 16S rRNA in summer were significantly higher than that in winter ($p<0.01$ for ARGs, $p<0.05$ for 16S rRNA). The absolute abundance of 16S rRNA in this study was lower than those in the Qingcaosha Reservoir (Shanghai) (1.4×10^5 – 1.1×10^8 copies/mL for water, 1.8×10^8 – 1.8×10^{11} copies/g for sediment) and higher than that in drinking water source of Yuqiao Reservoir (Tianjin) (10^9 copies/L) [40, 41]. The absolute abundance of ARGs was also lower than that in the Qingcaosha Reservoir (3.7×10^3 to 1.4×10^6 copies/mL for water, and 2.1×10^7 to 4.4×10^9 copies/g for sediment) and comparable to the abundance of ARGs in drinking water sources in China in previous study (1.68×10^5 to 2.31×10^7 copies/100 mL) [40, 42]. Meanwhile, it was observed that the absolute abundance of ARGs in water samples were significantly higher than that in sediment samples ($p<0.05$), but the absolute abundance of 16S rRNA in water and sediment samples was not significant difference ($p>0.05$). The dominant ARGs in water and sediment samples were multidrug, aminoglycoside and tetracycline resistance genes, with total average absolute abundance of 1.03×10^9 , 6.28×10^8 and 1.47×10^8 copies/L for water samples, followed by 2.76×10^8 , 1.00×10^8 and 2.87×10^7 copies/g for sediment samples, respectively. The result was consistent with that of the Karst River [16]. Previous studies on ARG pollution in large Chinese lakes (such as Dongting, Taihu, Chaohu, Honghu and Bosten Lake) showed that the sulfonamide, tetracycline, multidrug and aminoglycoside resistance genes were the most commonly observed in the aquatic environment [28, 32, 43, 44]. The different types and abundance of detected ARGs in the aquatic environment worldwide may be depend on



antibiotic usage patterns and bacterial species in different regions [44–46].

Characterization of MGEs

In the current study, a total of 51 MGEs were detected in the 55 target MGE subtypes (Additional file 1: Table S5). The total number of detected MGEs in the fw, fs, dw and ds samples were 50, 38, 39 and 34, respectively, and in water and sediment samples were 51 and 39, respectively. The number of MGEs was higher in summer than in winter, which was also consistent with the results of the ARGs (Fig. 2), and the corresponding absolute abundances of MGEs are shown in Fig. 3. The highest MGE abundance (total 2.39×10^8 copies/g) was detected in the fs6 sample location. The absolute abundance of MGEs was significantly higher in the flood season water samples than in the dry season water samples ($p < 0.01$), but the absolute abundance of MGEs had no

significant difference between sediment and water samples ($p > 0.05$).

Specifically, the 51 MGE subtypes detected include integrase genes *intI1* (class I integrase), *intI2* (class II integrase) and *intI3* (class III integrase), transposase genes (e.g., *tnpA-7*, *tnpA-1*, *tnpA-4*, *tnpA-5*, *tnpA-6*, *tnpA-3*, *IS26*, *IS91*, *TP614*, *IS256*, *IS200-2*, *IS3*, *IS6100*, *IS613*, and *IS1247*), plasmids (e.g., *IncN_korA*, *IncP_oriT*, *IncN_oriT*, *IncN_rep*, *IncHI2-smr0018*, *IncI1_repI1*, *IncW_trwAB*, *IncF_FIC*, *IncQ_oriT*).

Bacterial communities in the Douhe Reservoir samples

Diversity and richness of bacterial communities

Among all the samples collected from the Douhe Reservoir, the average Shannon index was 3.63 in the fw1–fw18 samples, 3.98 in the dw1–dw18 samples, 6.18 in the fs1–fs10 samples and 6.49 in the ds1–ds10 samples, respectively, see Additional file 1: Table S7. In addition, the

richness and diversity of the bacterial communities in the sediment samples were significantly higher than those in the water samples of two seasons, which were indicated by the Chao1 (Welch t test, $p < 0.01$) and Shannon (Welch t test, $p < 0.01$) indices, respectively. Meanwhile, the richness and diversity had significant difference between the sediment samples of summer and winter, based on the Shannon and Chao1 indices ($p < 0.01$). However, there was no comparable significant difference in the water samples of the two seasons ($p > 0.05$).

Composition of bacterial communities in samples of the Douhe Reservoir

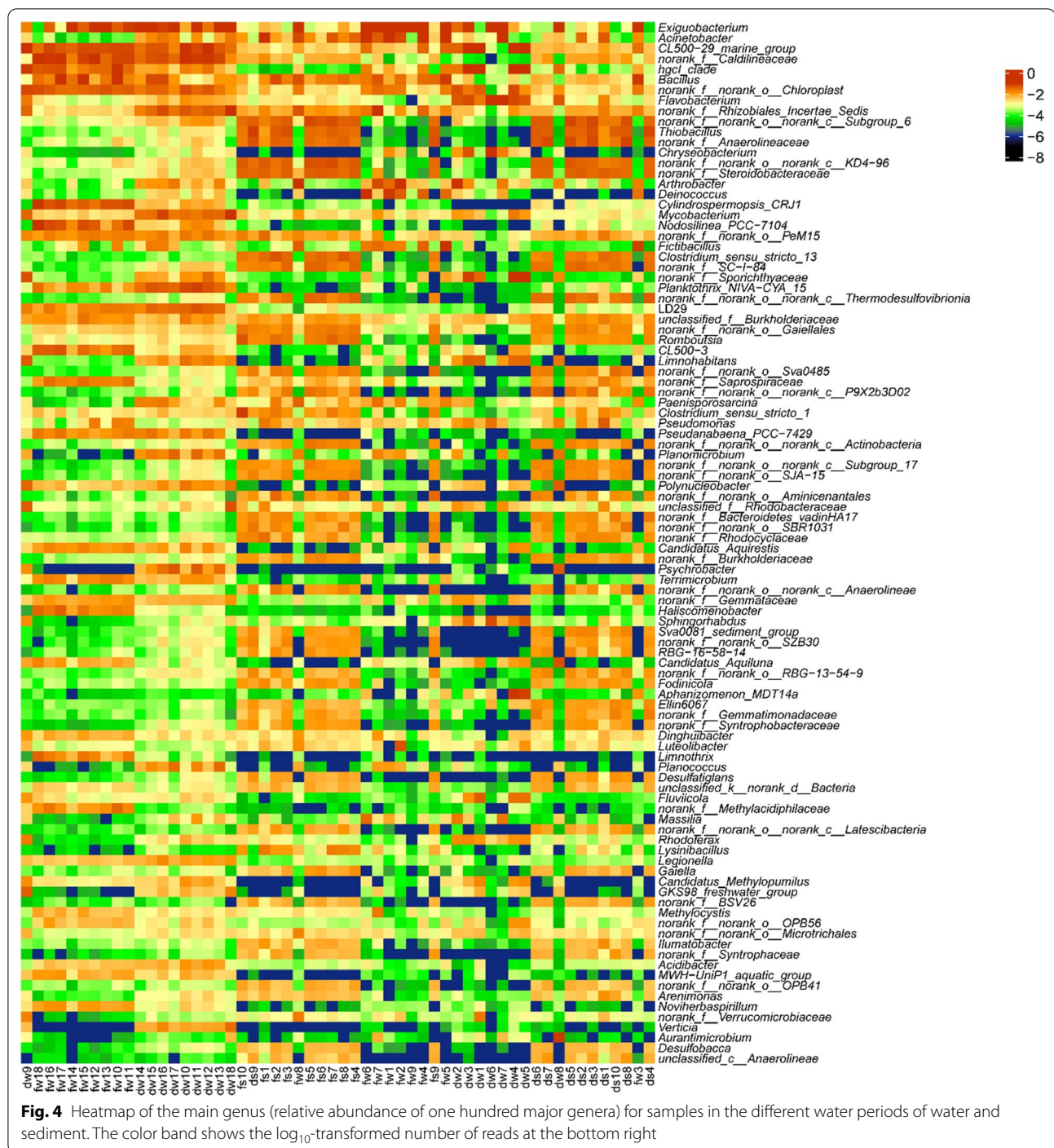
There were about 53 phyla, 163 classes, 402 orders, 640 families and 1,220 genera identified from the total of 36 water samples (including fw1–fw18 in summer and dw1–dw18 in winter), respectively, and there were about 64 phyla, 188 classes, 444 orders, 698 families and 1,229 genera identified from the total of 20 sediment samples (including fs1–fs10 in summer and ds1–ds10 in winter), respectively. The compositions of the bacterial communities of the water and sediment samples at the phylum and genus levels are shown in Additional file 1: Fig. S1 and Fig. 4, and the detailed data of percents of community abundance at the phylum and genus levels see Additional file 1: Table S8. At the phylum level of water samples (Additional file 1: Fig. S1), *Firmicutes*, *Proteobacteria*, *Actinobacteriota*, *Bacteroidota*, *Cyanobacteria* and *Chloroflexi* were the six major phyla, and the *Firmicutes* was overwhelmingly dominant for the largest contribution (with the average of 30.87%), followed by *Proteobacteria* (18.90%), *Actinobacteriota* (18.39%) and *Bacteroidota* (9.83%). Less abundant phyla were *Cyanobacteria* (9.61%) and *Chloroflexi* (4.17%). In the Karst River (Guizhou), *Proteobacteria*, *Bacteroidetes* and *Cyanobacteria* were the most abundant bacterial phyla [16]. Most studies have shown that the *Firmicutes*, *Proteobacteria*, *Bacteroidota*, and *Actinobacteriota* were the dominant composition of the bacterial communities at the phylum level of rivers, reservoirs and domestic waste water in many regions of the world [21, 28, 47–49]. At the genus level of the water samples of the Douhe Reservoir (Fig. 4), *Exiguobacterium*, *Acinetobacter*, *CL500-29_marine_group*, *norank_f_Caldilineaceae*, *hgcl_clade*, and *Bacillus* were the six dominant genera, and the *Exiguobacterium* was overwhelmingly dominant for the largest contribution (average of 24.14%), followed by *Acinetobacter* (6.46%), *CL500-29_marine_group* (5.02%), and *norank_f_Caldilineaceae* (3.64%). Less abundant genus was *hgcl_clade* (3.30%) and *norank_f_norank_o_Chloroplast* (3.10%). At the phylum level of sediment samples (Additional file 1: Fig. S1), *Proteobacteria*, *Chloroflexi*, *Firmicutes*, *Acidobacteriota*,

Actinobacteriota and *Bacteroidota* were the six dominant phyla, and the *Proteobacteria* was the greatest contribution (average of 25.55%) to the composition of the bacterial community, followed by *Chloroflexi* (16.62%), *Firmicutes* (17.77%) and *Acidobacteriota* (9.51%). Less abundant phyla were *Actinobacteriota* (9.10%) and *Bacteroidota* (5.92%). At the genus level of sediment samples (Fig. 5), *Thiobacillus*, *norank_f_Anaerolineaceae*, *norank_f_norank_o_norank_c_KD4-96*, *norank_f_Steroidobacteraceae*, *norank_f_norank_o_Vicinamibacterales* and *Clostridium_sensu_stricto_13* were the six major genera, and the *Thiobacillus* was the most abundant genus (average of 3.97%) of the bacterial community, followed by *norank_f_Anaerolineaceae* (3.79%), *norank_o_norank_c_KD4-96* (3.77%) and *norank_f_Steroidobacteraceae* (3.52%). Less abundant phyla were *norank_f_norank_o_Vicinamibacterales* (2.85%) and *Clostridium_sensu_stricto_13* (2.61%).

Difference of bacterial community of all the samples in the Douhe Reservoir

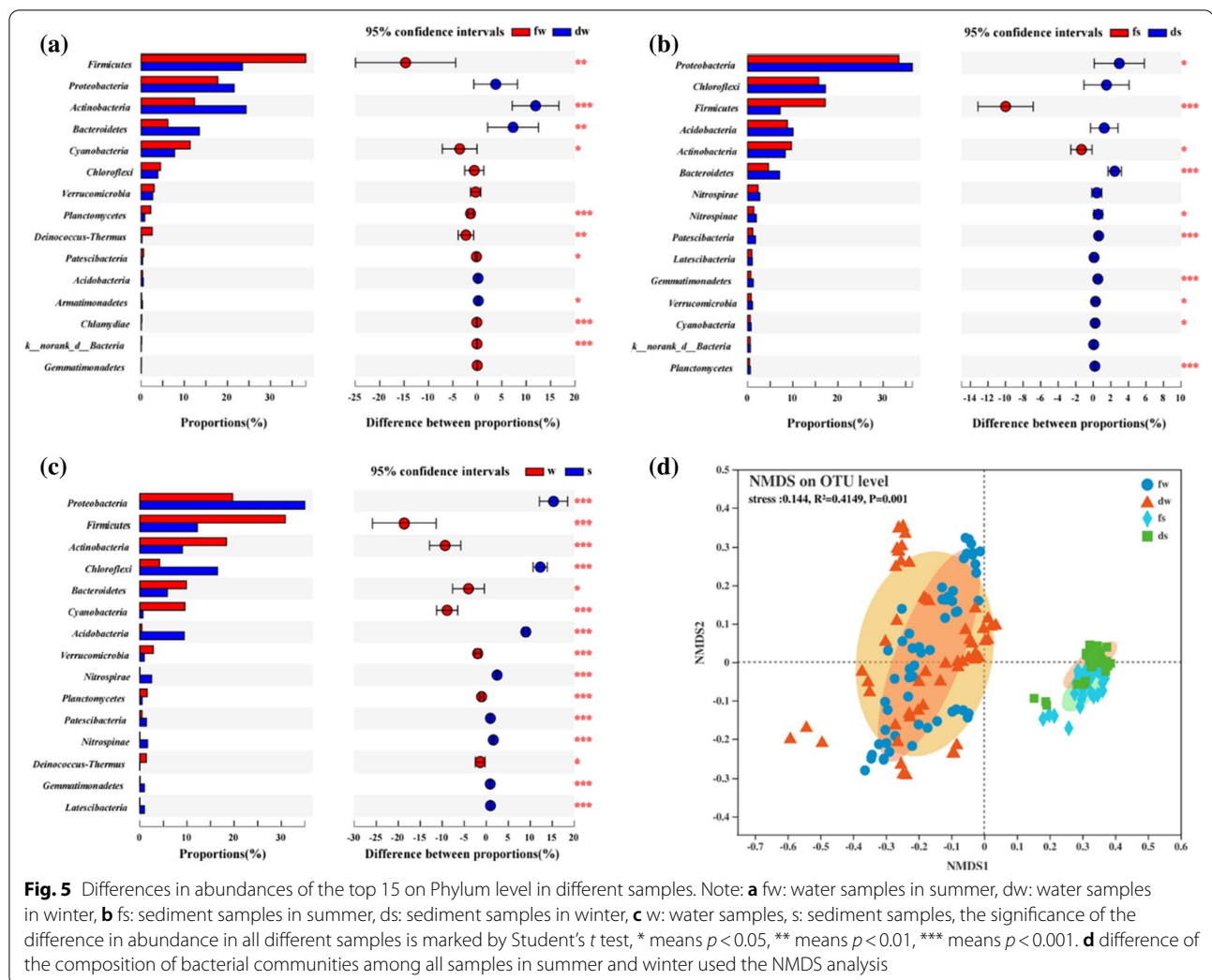
At the OTU level, the NMDS was performed to analyze the differences of the bacterial community distribution (Fig. 5d). It can be seen from Fig. 5d that the similarity of the bacterial communities composition of the fw1–fw18 and dw1–dw18. It also can be seen that the similarity of the bacterial communities composition of the fs1–fs10 and ds1–ds10, despite difference in their water period. By contrast, the Bray–Curtis distance of the water samples cluster was significantly different from that of the sediment samples cluster (Adonis, $p = 0.001$, Fig. 5d), which indicated that the clustering mode of the bacterial community was mainly affected by the sample type (water or sediment), little affected by the water period.

The differences between the composition of bacterial community on the phylum level identified in water and sediment samples in summer and winter were analyzed (Fig. 5). The proportions of *Firmicutes*, *Planctomycetes*, *Deinococcus-Thermus*, and *Chlamydiae* in the water samples of summer were significantly higher than those in winter samples, while the proportions of *Actinobacteria* and *Bacteroidetes* in water samples of winter were significantly higher than that in summer samples ($p < 0.01$, Fig. 5a). For the sediment samples, the proportions of *Firmicutes* and *Actinobacteria* in the summer samples were significantly higher than those in winter samples, while the proportions of *Bacteroidetes*, *Patescibacteria*, *Gemmatimonadetes* and *Planctomycetes* in the winter samples were significantly higher than those in summer samples ($p < 0.01$, Fig. 5b). It can be seen the differences between water and sediment samples from Fig. 5c that



the proportions of *Firmicutes*, *Actinobacteria*, *Cyanobacteria*, *Verrucomicrobia* and *Planctomycetes* in water samples were significantly higher than those in sediment samples, in contrast, the proportions of *Proteobacteria*, *Chloroflexi*, *Acidobacteria*, *Nitrospirae*,

Nitrospinae, *Gemmatimonadetes* and *Latescibacteria* in sediment samples were significantly higher than those in water samples ($p < 0.01$).



Factors that shape the distribution of ARGs and MGEs

Correlation between bacterial and ARGs vs MGEs

Most previous studies have indicated that the composition of bacterial community might influence the occurrence and abundance of ARGs and MGEs in aquatic and soil environments [50–53]. Due to the complexity of the correlation between bacterial community and ARGs in the Douhe Reservoir, the redundancy analysis (RDA) was used to reveal the correlation between bacterial community and ARGs (Fig. 6). Among the nine major bacterial genera in the Douhe Reservoir, *norank_f_Caldilineaceae* (Monte carlo permutation tests, $p = 0.002$, phylum of the *Chloroflexi*), *Cylindrospermopsis_CRI1* ($p = 0.122$, phylum of the *Cyanobacteria*), *Bacillus* ($p = 0.284$, phylum of the *Firmicutes*), *hgcI_clade* ($p = 0.184$, *Actinobacteriota*) and *CL500-29_marine_group* ($p = 0.314$, both the phylum of the *Actinobacteriota*) were the five significant microbial genera that affected the abundance of ARGs in the water of

summer (flood period) (Fig. 6a). In water of winter (dry period), *Flavobacterium* ($p = 0.161$, phylum of the *Bacteroidota*), *norank_f_Chloroplast* ($p = 0.159$, phylum of the *Cyanobacteria*), *norank_f_Rhizobiales_Incertae_Sedis* ($p = 0.158$, phylum of the *Proteobacteria*) were the three significant bacteria at the genus level that impact the abundance of ARGs (Fig. 6b). In the sediment samples of summer and winter, *norank_f_SC-I-84* ($p = 0.012$, phylum of the *Proteobacteria*), *Thiobacillus* ($P = 0.058$, phylum of the *Proteobacteria*) and *Acinetobacter* ($p = 0.454$, phylum of the *Proteobacteria*) and *norank_f_SC-I-84* ($p = 0.008$, both the phylum of the *Proteobacteria*), *norank_f_Vicinamibacteriales* ($p = 0.096$, phylum of the *Acidobacteriota*), *fnorank_f_Thermodesulfovibrionia* ($p = 0.184$, phylum of the *Nitrospirota*) were the three major bacteria at the genus level that influenced the abundance of ARGs in the sediment of flood and dry periods, respectively (Fig. 6c, d). These correlations between ARGs and bacterial

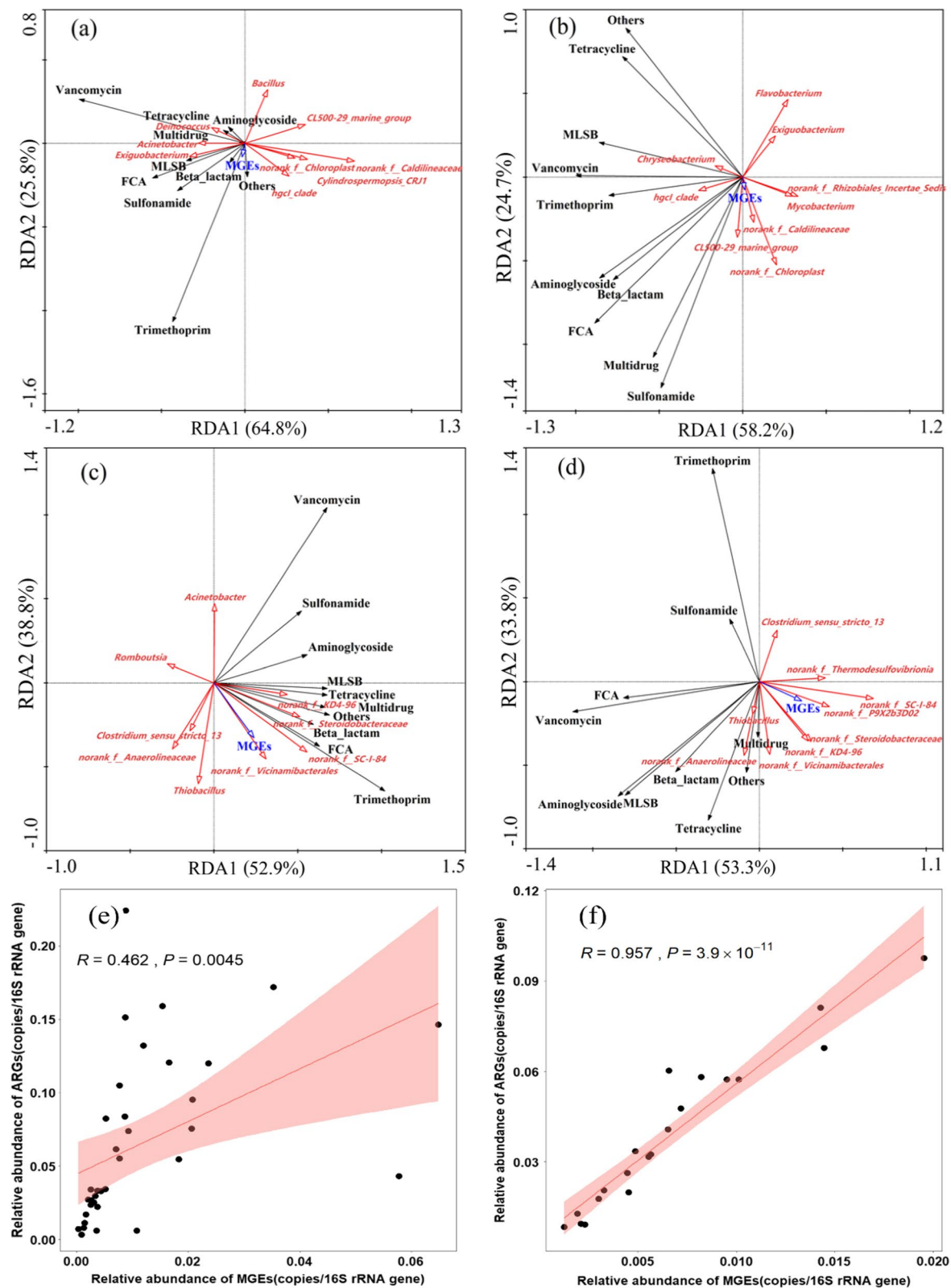


Fig. 6 RDA of the ARGs, MGEs and bacterial (Nine dominant bacterial genera) in samples. Water samples in summer (a) and winter (b), sediment samples in summer (c) and winter (d). OLS regression analysis between ARGs and MGEs for water (e) and sediment (f). Red areas show 95% confidence intervals

communities at genus level indicated that genus was the major factor that influence the occurrence, distribution and abundance of ARGs.

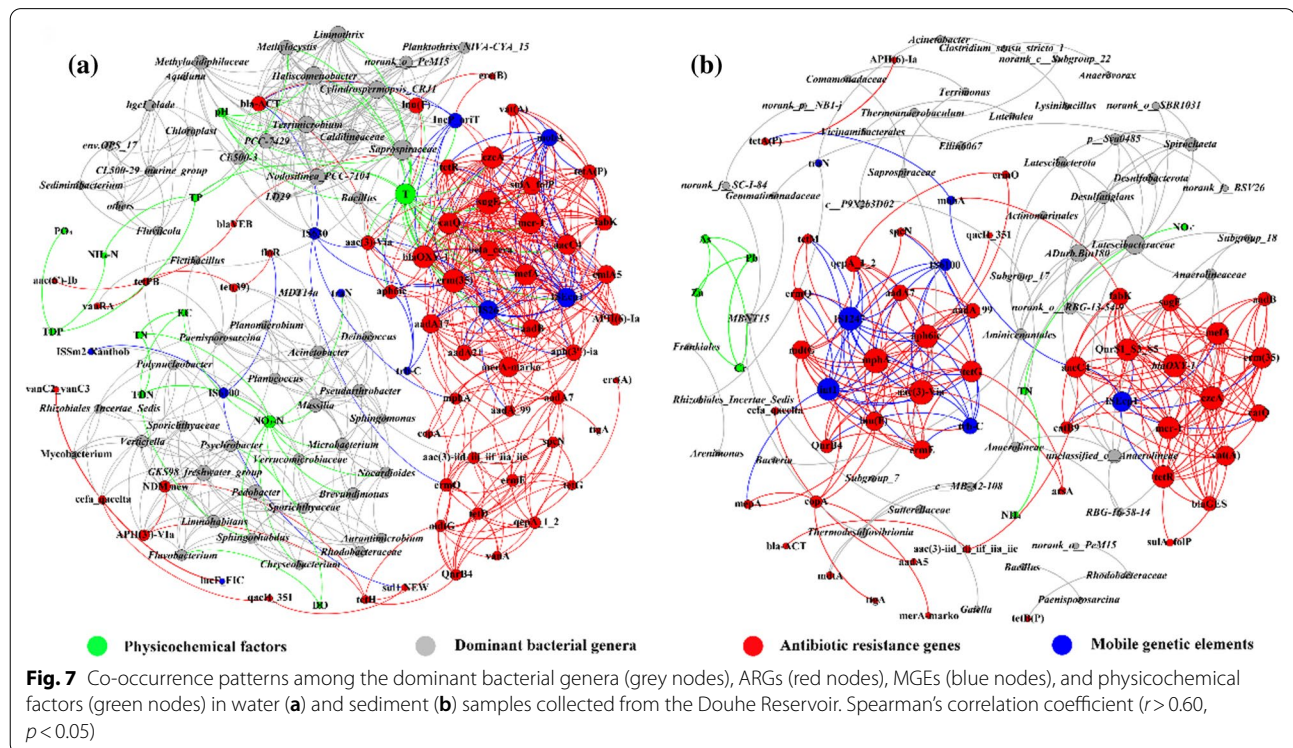
There are 19 transposase genes (including *tapA*-7, *tnpA*-1, *tapA*-4, *tapA*-6, *IS26*, *IS91*, *TP614*, etc.) and 3 integrase genes (including *intI1*, *intI2* and *intI3*) were detected among 51 MGEs, which indicated that the widespread of MGEs in antibiotic-polluted the Douhe Reservoir. Furthermore, the prevalence of *tnpA* gene could accelerate the abundance of ARGs through HGT. OLS was applied to reveal the correlation between ARGs and MGEs (Fig. 6e, f), it can be seen from Fig. 6e, f that there exist a positive liner correlation between ARGs and MGEs in the water ($R=0.462$, $p=0.0045$) and sediment samples ($R=0.957$, $p=3.9 \times 10^{-11}$). It indicates that the HGT as a main mechanism is likely to have occurred in the water and sediment for the widespread distribution of ARGs of the Douhe Reservoir and the MGEs may play a role in the persistence and proliferation of related resistance phenotypes [54, 55].

Network analysis among physicochemical factors, microbial communities, MGEs and ARGs

The co-occurrence network analysis was used to investigate the relationship between the major bacterial genera, environmental physicochemical factors, MGEs and major ARG subtypes based on the ARGs' relative

abundance (Fig. 7), and in each network analysis, the Spearman's correlation coefficient was greater than 0.6 ($p < 0.05$). Furthermore, we hypothesized that if there was a significantly positive correlation among ARG subtypes and coexisting bacterial genera, MGEs and the environmental physicochemical factors of the samples, then the co-occurrence pattern between ARGs and different types of bacterial genera could be used to provide potential host information for ARGs, and obtain the main drivers of ARGs based on their correlation with bacterial genera and environmental factors.

Many studies have shown that the bacterial communities can significantly affect the distribution of ARGs and may act as hosts of ARGs, some bacteria can even synthesize antibiotics [56, 57]. Hence, the composition of the bacterial community was studied based on the heatmap of community and NMDS analysis (Figs. 4 and 5), and it can be concluded that the bacterial community has a significant difference in temporal (two seasons) and spatial (water and sediment) distribution. Especially, the opportunistic pathogens in tap water mostly come from drinking water sources and may pose risk to human health and even cause diseases. In this study, it can be seen from Fig. 7 that the bacteria (genus-level) not only have a significant correlation with ARGs subtypes in water (Fig. 7a) and sediment (Fig. 7b), but also have a significant correlation with physicochemical factors. However, there



showed more significantly positive correlations between the bacteria genera and ARG subtypes in water samples than in sediment samples. For instance, in water samples (Fig. 7a), *Acinetobacter* (phylum of the *Proteobacteria*) was the possible host of some major ARGs such as multidrug-resistance genes (e.g., *floR*), tetracycline (*tet(39)*), *Caldilineaceae* (phylum of the *Chloroflexi*) was the possible host of Beta-lactam-resistance genes (*bla-ACT*), MLSB-resistance genes (*lnu(F)*) in the Douhe Reservoir. Likewise, the *Bacillus* (phylum of *Firmicutes*) may be the potential host of FCA (*catQ*), Beta-lactam (*beta_ccra* and *blaOXY-1*), MLSB-resistance genes (*mefA*) and multidrug-resistance (*czcA*). Furthermore, a total of 25, 21, 11, 7, and 5 genera affiliated with *Proteobacteria*, *Firmicutes*, *Bacteroidota*, *Actinobacteriota*, and *Cyanobacteria*, respectively, were positively correlated with some ARGs and MGEs. Han [58] have also demonstrated that *Bacillus* was significantly correlated with some ARGs, and *Proteobacteria*, *Actinobacteria*, *Chloroflexi* were significantly correlated with some ARGs and MGEs in many drinking water sources of cities of China. Furthermore, *Acinetobacter* and *Bacillus* belong to opportunistic pathogens which have been observed in many drinking water sources. The results indicating that opportunistic pathogens might play role of host of ARGs. It is worth noting that *Acinetobacter* can cause pneumonia and has potential to antimicrobial resistance [15]. Hence, opportunistic pathogens carry ARGs may pose risks to human health.

In sediment samples (Fig. 7b), both *Thermodesulfovibrionia* (phylum of *Nitrospirota*) and *MB-A2-108* (phylum of *Actinobacteriota*) were the possible hosts of MLSB resistance genes (*mdtA*), *Rhodobacteraceae* (phylum of *Proteobacteria*) was also found to be the potential host of tetracycline resistance genes (*tetB(P)*). *Acinetobacter* and *Rhizobiales_Incertae_Sedis* (both phylum of *Proteobacteria*) were the potential host of MGE (*traN*). Especially, the *Nitrospirota* is widely present in polluted aquatic environment, as a kind of nitrite oxidizing bacteria, it plays an essential role in the nitrogen cycle of the microbial communities [59]. These correlations between bacterial genera and ARG types indicated a result that bacterial genera were the main driver of ARG distribution pattern in water and sediment of reservoirs, which has been demonstrated in previous studies of water of the Karst River and Poyang Lake, and sediment of the Ebinur Lake [16, 38, 60]. These results also indicate that the host bacterial communities of ARGs are different in the environmental gradients. In addition, it should be noted that network co-occurrence is only a mathematical statistical analysis based on correlation testing, and its reliability in predicting potential host bacteria for ARGs will be affected. To ensure the safety of drinking water sources, there is an urgent need for more substantial

investigations on opportunistic pathogen of bacteria that may carry ARGs, such as molecular detection of ARG-host.

Many studies have demonstrated that the HGT between bacterial communities mediated by MGEs can accelerate the spread and increase the abundance of ARGs in the environment [61–63]. Hence, MGEs were strongly correlated with ARGs and bacterial communities because of their significant effect on driving ARGs among various microorganisms in environment [64]. For instance, integrase *intI1* as a good represent for the anthropogenic pollution has been demonstrated that have key influence in the occurrence of ARGs, and is widely used in environmental investigation [15, 65]. In this study, it can be seen from Fig. 7 that some relative abundance of MGEs (*IS26*, *ISEcp1* and *mobA*) and MGEs (*IS1247*, *intI1* and *ISEcp1*) showed the strong correlations (Spearman's $r=0.65\text{--}0.96$ and $0.61\text{--}0.92$, $p<0.05$) with ARGs in the water and sediment samples, respectively, which indicate that the occurrence and prevalence of MGEs play an important role in the abundance of ARGs in the water and sediment environment. Specifically, 22 detected ARG subtypes belong to almost all types of ARGs (e.g., aminoglycoside, MLSB, FCA, multidrug, and tetracycline resistance genes) were significantly related to *intI1* ($p<0.05$), which indicates that *intI1* played a key role in the proliferation and dissemination of many types of ARGs. Wang et al. [38] also found a similar phenomenon that *sul1* and *sul2* were significantly positively correlated with *intI1* ($p<0.01$) in samples. Nevertheless, we also found that the co-occurrence patterns between ARGs and MGEs were obviously different between water and sediment samples (Fig. 7a, b), this indicated that the proliferation and spread of ARGs by the MGEs may be influenced by the different environment medium. In particular, the influence on ARGs by MGEs in the water was much stronger than that of in sediment, resulting the same spatial distribution characteristic of ARGs and MGEs (details described in Figs. 2 and 3).

In addition to bacterial communities, environmental factors may also influence the occurrence and fate of ARGs, studies have demonstrated the environmental factors, such as T, pH, EC, $\text{NH}_4\text{--N}$ and $\text{NO}_3\text{--N}$ present positive correlation with ARGs [15, 16]. In current study, the environmental physicochemical factors (including T, $\text{NO}_3\text{--N}$, TDN and TP) mostly present positive correlation with ARGs and bacterial genera in water samples, and merely a few physicochemical factors had correlations with the ARGs and bacterial genera in sediment samples, which means that the physicochemical factors had a certain impact on the composition of bacterial communities and the occurrence of ARGs in the Douhe Reservoir. Besides, it also

can be seen from Fig. 7 that some environmental factors, such as TDN, NO₃-N, T and pH were positively correlated with some bacterial genera (*Bacillus*, *Cylindrospermopsis*, *CRJ1*, *Saprospiraceae*, *Nodosilinea*, *PCC-7104*), indicating that these bacteria may use TDN and NO₃-N as the major nutrients and energy for their own growth and metabolism or may be affected by temperature and pH. Xiao [66] confirmed that *Bacillus* is resistant to heavy metals and participates in nutrient cycling. This indicates that environmental factors may affect the occurrence of ARGs by affecting bacterial community. In addition, studies also have shown that there is a significant correlation between the content of heavy metals in sediments and the abundance of ARGs, indicating that heavy metal pollution may be a major factor affecting the abundance of ARGs. Zhao [67] have confirmed that the collaborative screening of nutrients and heavy metals played an important role in the dynamic changes of ARGs in sediments by analyzing the relationship between ARGs and heavy metals and nutrients in the Yellow River Delta sediments. Overall, the network analysis results about the correlations among physicochemical factors, microbial communities, MGEs and ARGs further confirmed that the fate of ARGs is closely related to their potential host bacteria [68], and the physicochemical factors may impact the composition of bacterial communities.

Conclusions

This study revealed the characteristics of ARGs, MGEs, and bacterial communities of water and sediment in the drinking water sources of the Douhe Reservoir in summer and winter. There were significant differences in the structure of bacterial communities and profiles of ARGs and MGEs between the water and sediment samples of the two seasons, but there were no differences of the major types of ARGs between the water and sediment samples in winter and summer. Six classes of MGE subtypes (*IS26*, *ISEcp1* and *mobA* for water, *IS1247*, *intI1* and *ISEcp1* for sediment) played an important role in the spread of ARGs. The evolution of bacterial communities has the most direct contribution to changes in ARGs. Furthermore, the variation of bacterial communities, ARGs and MGEs in drinking water sources of the Douhe Reservoir appeared to be related to the environmental factors, particularly of the values of T, NO₃-N, TDN and TP ($p < 0.05$), and the further research is necessary. Overall, the results of this research will help us understand the ARGs in the drinking water sources and provide us essential information to improve the management strategies for reservoir.

Abbreviations

ARGs: Antibiotic resistance genes; MGEs: Mobile genetic elements; NO₃-N: Nitrate-nitrogen; STPs: Sewage treatment plants; OTUs: Operational taxonomic units; TDN: Total dissolved nitrogen; MLSB: Macrolides-lincosamides-streptomycin B; TP: Total phosphorus; NH₄-N: Ammonia nitrogen; T: Temperature; HT-qPCR: High-throughput quantitative PCR; EC: Electrical conductivity; HGT: Horizontal gene transfer; DO: Dissolved oxygen; FCA: Fluoroquinolone, quinolone, florfenicol, chloramphenicol, and amphenicol; NMDS: Nonmetric multidimensional scaling; OLS: Ordinary least squares; RDA: Redundancy analysis; HCO₃⁻: Bicarbonate ions; qPCR: Quantitative polymerase chain reaction.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12302-022-00635-x>.

Additional file 1: Table S1. GPS location of sampling locations. **Table S2.** Water quality of the selected samples. **Table S3.** Sediment quality of the selected samples. **Table S4.** Primer sets used in this study. MLSB (Macrolide-Lincosamide-Streptogramin B), MGEs (mobile genetic elements) and FCA (fluoroquinolone, quinolone, florfenicol, chloramphenicol, and amphenicol). **Table S5.** Relative mean abundance of each ARG and MGE subtypes in sample (copies/16S rRNA gene). **Table S6.** Average number of detected ARGs and MGEs of the each sample. **Table S7.** Diversity indices of bacterial communities in each sample. **Table S8.** Bacterial phyla percent in each water samples. Bacterial genus percent in each water samples. Bacterial phyla percent in each sediment samples. Bacterial genus percent in each sediment samples. **Figure S1.** Relative abundance of different bacterial phyla in water and sediment samples.

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Author contributions

KZ: writing—original draft; YF: supervision, methodology; SC: resources, funding acquisition; QF: visualization, data curation; QZ: project administration; GY: software, data curation; XS: reviewed drafts of the paper. All authors read and approved the final manuscript.

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Availability of data and materials

The complete data set of this study is included within the article and the Additional file.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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